Vitamin D for Autism Spectrum Disorders:a Protocol for Systematic Review and Meta-analysis

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Abstract

Introduction

Autism spectrum disorder(ASD) is a neurodevelopmental disorder characterized by repetitive and stereotypic behavior, impaired social interactions and communications. An increasing number of reseachers suggested that vitamin D may be participated in the pathogenesis of ASD, and vitamin D deficiency may be one of the causes of ASD.Meanwhile, some studies have shown that vitamin D can improve the core symptoms in ASD children.On top of updating the latest systematic review on vitamin D supplementation and ASD, we aim to conduct the meta-analyses of trials on vitamin D3 supplementation and ASD. Besides, we will conduct for the first time subgroup analyses based on individual patient data collected from randomised controlled trials.

Methods and analysis

A systematic review and individual patient data meta-analysis on randomised placebo-controlled trials with a vitamin D3 intervention. All studies are searched from inception without time restriction. The addressed outcomes are

25-hydroxyvitamin D, Childhood Autism Rating Scaleas(CARS), Autism Behavior Checklist (ABC), Social Responsiveness Scale(SRS) and M-CHAT. Trial results will be reanalysed using Cox proportional hazard regression models and meta-analyses are performed. Cochran's Q-Test and the I² index will be used to statistically assess the level of heterogeneity, while sensitivity and subgroup analyses serve to identify potential causes of heterogeneity. Subgroup analyses will be conducted for age, sex, 25-hydroxyvitamin D level,vitamin D3 dosing, follow-up time,and the core symptoms of ASD. Publication bias will be assessed by funnel plots and Egger's test.

Ethics and dissemination

Ethical approval is not required since no human beings are involved in this systematic review. Results will be published in a peer-reviewed journal with open access.

Strengths and limitations of this study

1.Meta-analysis on vitamin D and ASD,we not only study the relationship between vitamin D deficiency and the occurrence of ASD, the preventive and therapeutic effects of vitamin D on ASD, but also the influence factors of vitamin D supplementation on the therapeutic effect of ASD. 2.Assess study quality, sources of heterogeneity and bias minimise the risk of bias and will gather reproducible results.3.Eligible data for meta-analysis may be limited.

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Background

Autism Spectrum Disorders(ASD) is a group of neurodevelopmental disorders of early childhood,ASD was first reported in 1943 by the American Doctor Kanner,And named "Early infantile autism ",total of 11 children.These children have abnormal in social communication,behavior and interests.¹ ASD was once considered rare disease, The prevalence of ASD has risen dramatically over the last several decades in various countries.² The World Health Organization pointed out that ASD has become one of the most rapidly increasing serious diseases in the world, and it is a major public problem that seriously affects the health of the population.^{3 4} The etiology and pathogenesis of ASD are unknown,and the interaction of genetic and environmental factors is thought to be involved in the occurrence of ASD,the specific pathogenesis still needs to be further studied.⁵ the cause of the prevalence of ASD rapid growth is not fully known. One can get clues from epidemiological data. Studies show ASD is more common in urban than rural areas, in cloudy and rainy areas, in areas that get the least solar UVB and in areas with high air pollution.⁶ As buildings, rain and clouds all reduce surface UVB (UVB is that spectrum of sunlight that triggers vitamin D production in the skin), these data are all consistent with the etiological hypothesis that vitamin D deficiency might cause ASD was first proposed by Cannell in 2008.^{7 8} Current studies have found that 1 α -hydroxylase, the key enzyme for vitamin D synthesis, and vitamin D receptor (VDR) are widely present in brain tissue, and vitamin D plays an important role in brain development.⁹ Vitamin D has important effects on brain development and function, including neuronal differentiation, proliferation and apoptosis, regulating synaptic plasticity, the ontogeny of the dopaminergic system, and reducing oxidative burden(Karras et al., 2018).¹⁰ more and more researches have suggested that vitamin D may be participated in the pathogenesis of ASD, and vitamin D deficiency may be one of the causes of ASD.¹¹ Meanwhile, some studies have shown that vitamin D can improve the core symptoms in ASD children.¹²

A number of randomised trials showed that ASD had lower vitamin D level in different countries and races,¹³⁻¹⁶ but several studies reached the opposite conclusions.¹⁷⁻¹⁹ Meanwhile, A prospective research found that neonatal vitamin D was associated with the occurrence of ASD,²⁰ however a cohort study revealed gravida with vitamin D deficiency at mid-gestation in high risk to give birth to ASD infants while neonatal vitamin D was not associated with ASD.²¹ A nested case-control study in China found the lower neonatal vitamin D was associated with a higher risk of ASD,²² but some studies from the United States did not support it.²³⁻²⁵

As mentioned above, more and more studies measuring the level of vitamin D with ASD , with some contradictions in the results.Moreover, several prospective studies performed on vitamin D levels before ASD diagnosis, also exists some paradoxical results. The level of vitamin D is affected by many factors, including diet,sunlight,physiological conditions.ethnicity,and genetic polymorphism, Researchers could not control all factors which affect vitamin D level. Meta-analysis can integrate the results of various studies and increase the sample size to reach a consistent and credibility conclusion.Therefore, there is a need for a systematic review that reanalyses individual patient data (IPD) from previous trials .we can through a meta-analysis of the case-control studies and prospective studies to investigate relationships

between ASD and vitamin D ,and to explore the potential reasons of heterogeneity between different studies.

Objective

The objective of our systematic review is to assess the the relationship between ASD and vitamin D ,including baseline 25(OH)D levels, ASD symptoms at baseline and compliance. Systematically review all studies on this relationship, including studies on the potential influence factors that underlie this relationship.The main outcomes include“25-hydroxyvitamin D”,“Childhood Autism Rating Scaleas(CARS)”,“Autism Behavior Checklist (ABC)”,“Social Responsiveness Scale(SRS)”and“M-CHAT”.These outcomes are universally used in ASD studies

and do not need our further refinement.

First of all, we will update the previous systematic reviews on vitamin D supplementation and ASD including newly published trials and unpublished data from trials by asking the authors for data on core of symptoms of ASD.Then, we intend to gather data for an IPD meta-analysis. Afterward, we plan to conduct IPD meta-analyses on vitamin D and core of symptoms of ASD. Fourth, we will conduct subgroup analyses to explore potential influence factors that underlie this relationship between ASD and vitamin D . The timetable for the review is shown in table 1.

Table 1 Proposed timetable for conducting the review

Step	Time frame for completion
Literature search	3 months
Data extraction and acquisition	2 months
Quality appraisal	2 months
Data analysis and meta-analysis	2.5 months
Writing of manuscript	2.5 months
Total	12 months

Methods And Analysis

eligibility criteria

All trials will be selected that have published on our research topic,and eligibility criteria were set according to PICOS : the participants (P), the interventions (I), the comparison (C), the outcome (O),and the study design (S).Only trials with eligible data for a meta-analysis will be included.

Step 1: inclusion criteria for trials

The Participants

Neonates, children or adolescents of ASD and pregnant women.

The Interventions

We will focus on trials that used vitamin D3 in any dose and any regimen as the intervention. However, we should pay attention to the time of the 25(OH)D supplementation,because it takes 3 to 6 months for 25(OH)D levels to reach homeostasis after initiating supplementation. Besides, we will also include studies using vitamin D3 bioequivalent substances .

The Comparison

We will include only studies, which diagnosed ASD without vitamin D supplementation as the comparator.

The Outcomes

To be eligible for inclusion in a meta-analysis trials need to have assessed the outcome of the level of 25(OH)D, the score of CARS,the score of ABC,the score of SRS and the score of M-CHAT. We also intend to record studies with the outcomes of ASD incidence and contact the authors if they have data for the outcomes needed for the planned meta-analyses in an intermediate step of our systematic review, The contents of outcomes are shown in table 2.

Table 2 The contents of outcomes

Outcome	contents
25(OH)D levels	testing serum 25(OH)D level
Childhood Autism Rating Scaleas(CARS)	CARS is a standardized diagnostic scale for ASD, which was compiled by E.Schopler, R.J.Reichler and B.R.Renner in 1980.It evaluates children with autism in 15 aspects and suitable for high risk children over 2 years old. ²⁶
Autism Behavior Checklist (ABC)	ABC, prepared by Krug in 1978, primarily to assess the severity and change of autism symptoms,There are 57 items in the scale, including five aspects: feeling, communication, body movement, language and self-care. ²⁷
Social Responsiveness Scale(SRS)	SRS was compiled in 2005, which is suitable for screening autism in children aged 4-18. The SRS questionnaire consists of 65 items and is divided into five dimensions: social perception, social cognition, social communication, social motivation and autism behavior; The total score is ≥60 points, indicating that the primary screening is positive. This scale is the first ASD screening tool to quantitatively score social functions. ²⁸
M-CHAT	M-CHAT is one of the main screening scales for ASD in the early stage, which is suitable for children between 16 months and 30 months. The scale includes 23 items and can screen children with ASD in the early stage. ²⁹

Study design

We will include randomised controlled trials (RCTs) , case–control, cohort, and nested case–control studies. No restrictions are applied for the form and time of vitamin D.Studies need to report the odds ratio (OR)/relative risk (RR) for ASD incidence,and mean and standard deviation of vitamin D concentration .We will exclude studies if involved participants were comorbid with any other disease that affect the level of vitamin D .

Step 2: inclusion criteria for pooling in meta-analysis

Studies will be included for pooling in the meta-analysis if the risk ratio and 95% CI for at least one outcome of interest 25(OH)D levels,the score of CARS,the score of ABC,the score of SRS and the score of M-CHAT) were reported. We will include the largest number of information in the meta-analysis when several publication reported the same trial.

Information sources and search strategy

We will search in MEDLINE, PubMed, EMBASE, Web of Science,and the Cochrane Library from database to identify studies on vitamin D and ASD. The search terms we used were medical subject headings(MeSH) phrases combined with text words relating to vitamin D (“vitamin D” OR “25 hydroxyvitamin d3” OR “d3,1,25 dihydroxyvitamin” OR “1,25 dihydroxyvitamin d3” OR “1,25 dihydroxy 20 epi vitamin d3”OR“cholecalciferol”) and autism (“autistic” OR “autism” OR “ASD”OR“Autism Spectrum Disorder”) . We will search for previous systematic reviews in the KSR Evidence and Cochrane Database of Systematic Reviews (CDSR, OVID interface).No restrictions were applied for the languages, time, and geographical position of the studies. The search string for MEDLINE is shown in table 3.

Table 3 Search string for MEDLINE

Step	Search string
1	'vitamin D'(tw) OR 'vitamin D'(MeSH) OR cholecalciferol*(tw) OR 'vitamin d 3'(tw) OR '25 hydroxyvitamin d3'(MeSH) OR'25 hydroxyvitamin d3'*(tw) OR 1,25 dihydroxyvitamin d3*(tw) OR 1,25 dihydroxyvitamin d3(MeSH) OR calcitriol(tw) OR '1,25 dihydroxy 20 epi vitamin d3'(tw) OR 1,25 dihydroxy 20 epi vitamin d3l(MeSH)
2	autistic(tw) OR autistic(MeSH) OR autism(MeSH) OR autism(tw) OR ASD(tw) OR ASDI(MeSH) OR Autism Spectrum Disorde(tw) OR Autism Spectrum Disorde(MeSH)
3	(((((((('randomized controlled trial'(pt)) OR 'controlled clinical trial'(pt)) OR randomized(tiab)) OR placebo(tiab)) OR randomly(tiab)) OR trial(tiab)) OR groups(tiab))) NOT ((animals(mh) NOT humans(mh)))
4	placebos(MeSH) OR placebo(tw)
5	2
6	1 AND 3 AND 4 AND 5

Data collection and management

Data were selected from eligible studies by two reviewers. They cannot know each

other's decision . References will be stored and managed by The EndNote software. The two reviewers will extract data include first author, year, country, study design, sample size, number of samples, sex, age, vitamin D measurement method, 25(OH)D levels, the score of CARS, the score of ABC, the score of SRS and the score of M-CHAT.

Quality assessment

Two independent reviewers will assess all studies according to the Newcastle–Ottawa Scale (NOS).³⁰ This NOS includes three aspects: study-participant; the comparability of study participants; the exposure or outcome of studies. The results fall into low-quality and high-quality . Two reviewers (WZQ and DR) independently

evaluated the eligible studies. Any discrepancies were resolved by discussion. If there is a disagreement, they will be discuss the key points until a consensus is reached.

Descriptive analysis and meta-analysis

Measures of treatment effect

The 25(OH)D levels and the score of scales be addressed by estimating the overall effect size and 95% confidence interval (95% CI). We will describe the difference of mean 25(OH)D levels between ASD and control groups by Mean difference (MD).

Data synthesis

Due to differences between the studies, all fit the following four major meta-analysis data will be composed deriving random effects results with the Laird

and DerSimonian method and fixed-effects summary estimates using the Mantel-Haenszel method

1. Association of vitamin D3 levels and ASD occurrence
2. study of the effect of vitamin D supplementation of ASD children
3. study of vitamin D supplementation during pregnancy ;
4. Outcomes including incidence of ASD, vitamin D levels, and the score of scales.

For the categorical variable, we will calculate the pooled OR/RR and 95% CI of eligible studies. we will perform a meta-analysis on case-control studies with OR if there are studies did not report vitamin D concentration but provided OR and 95% CI for the possibility of ASD which is vitamin D insufficiency or deficiency. To further explore the effect factors of the treatment by methodological or patient characteristics differences of the studies, we will performed subgroup analyses with eligible data.

Subgroup analyses

1. Age (0-1 years vs 1-3 years vs ≥ 3 years)
2. Sex (male vs female)
3. Vitamin D3 supplementation duration (<6 vs ≥ 6 months)
4. Vitamin D3 dosing (<1000 IU vs 1000–2000 IU vs >2000 IU/day).

Assessment of heterogeneity

We will measure heterogeneity by Cochran's Q test and I^2 statistic.. I^2

shows the ratio of heterogeneity, when $I^2 \geq 50\%$ indicated high heterogeneity. Meanwhile, We will perform subgroup and meta-regression analyses to explore sources of heterogeneity.

Assessment of publication bias

We will assess Publication bias by visually in funnel plots and tested for with Egger's test.

The quality of the body of evidence

We will evaluate the quality of all outcomes by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.

Patient and public involvement

Since this is a systematic review scheme, no patients and participants need to be recruited.

Ethics and dissemination

This systematic review is a summary of already published data. All studies had ethics approvals, so, we don't need an ethics approval.

Discussion

We will study the relationship between vitamin D and ASD by this systematic review. We will not only study the relationship between vitamin D deficiency and the occurrence of ASD, the preventive and therapeutic effects of vitamin D on ASD, but also the influence factors of vitamin D supplementation on the therapeutic effect of ASD.

This research protocol urged us to think carefully all the details and potential problems during the systematic review. Possible limitation of this review include a potentially high heterogeneity due to seasons, geographical position, measurement methods of vitamin D, different definitions of vitamin D deficiency and follow-up.

To guarantee that the research results are meaningful to clinicians and patients, we will assess the quality of the data of included studies. Studies have found that vitamin D deficiency is common in ASD, but the preventive and therapeutic effects of vitamin D on ASD are not yet clear. If the planned systematic review determines the effect of vitamin D supplementation on ASD, and taking into account the high prevalence of vitamin D deficiency in children and adolescents with ASD, pregnant women and breastfeeding women, we will recommend vitamin D testing and appropriate supplementation for this group of people. At the same time, it is recommended that researchers consider vitamin D-related factors as potential prevention and treatment measures for ASD. Compared with other current treatment methods for ASD, the cost of vitamin testing and supplementation is very low, and vitamin D supplementation will be highly cost-effective.

Declarations

contributorship statement

All authors made substantial contributions to the systematic review, YL, JW and XC are responsible for the systematic review. HYH and XMH arranged the search methods and the risk of bias assessment strategy. YC, SYY, MW and ZZ planned the inclusion criteria and the statistical methods. JW and XC drafted the protocol publication, which YL and DDQ revised critically. All authors approved the final version to be published.

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Competing interests None declared.

Data sharing statement Extra data is available by emailing liuyun@etyy.cn.

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